1. Viruses are obligate parasites that redirect the host cell machinery towards synthesis of their gene products and replication. In order to overcome the restriction imposed for the use of eukaryotic translation system, viruses have evolved different strategies to express their genes. Further, they have developed strategies that enable expression of maximum number of functional proteins from their limited genetic material.

2. Unlike in animal viruses, which can negotiate their entry into the host cell by manipulation of host's array of receptor systems, the plant viruses face the impervious barrier of the cell wall. The primary infection of plant viruses is some time confined to a single cell or a few cells, which occurs after mechanical damage to the cell wall and plasma by the vectors that transmit the virus or by mechanical inoculation. The infection is passed on to the adjacent cells with the help of viral encoded MPs.

3. The specificity of a plant virus infection does not occur at the level of replication, as plant viruses have the ability to replicate within cells of non-host species as well. The susceptibility is linked to the ability of the virus to evolve functions that enables it to gain access to the phloem, the long distance transport system, and thereby spread as a systemic infection.

4. Though there have been many classifications of MPs, the sequence conservation is very little among the MPs. However, irrespective of the super families to which MPs belong, they have some basic underlying similarities in functional domains. At least three domains have been identified in most of the MPs of RNA viruses. These are a domain for RNA binding, a domain for cooperative RNA binding, and a domain for interaction with plasmodesmata and other ancillary proteins required for movement.

5. There are two well established mechanisms by which MPs translocate the viral cargo across the plasmodesmata. One, by the formation of RNP complex, called the M complex, containing the MP, viral genomic RNA, and ancillary proteins which translocates to the plasmodesmata, increases the size exclusion limit and

passes through it, as well studied in TMV type movement proteins. The other mechanism is by the formation of tubules through which the intact virion passes from cell to cell, as observed in the case of CPMV.

6. *Sobemovirus* is a floating genus, not assigned to any family. This genus is named after the type member, *Southern bean mosaic virus* (SBMV). According to the present ICTV database there are 13 documented and 4 tentative *Sobemoviruses* species.

7. The present thesis deals with the mechanism of movement process in *Sesbania mosaic virus* (SeMV). SeMV infects *Sesbania grandiflora* belonging to *Fabaceae* and is native to Andhra Pradesh, India. It is a single-stranded positive sense RNA virus with a genome length of 4149 nucleotides. The genome encodes four potential overlapping open reading frames (ORFs). ORF1 codes for an 18.4 kDa protein that is a putative movement of the virus. The ORF2a and 2ab encode for two polyproteins (single polypeptide chain having more than one functional protein). The 2ab protein is expressed as a *trans*-frame polyprotein, translation brought about by ribosomal frame shifting mechanism. ORF 3 present at the 3' end of the genome codes for CP. CP is responsible for the encapsidation and protection of the viral genome. The capsids of SeMV are made of 180 copies of CP subunits that are built in an icosahedral geometry with T=3 symmetry. The mechanism of SeMV assembly and polyprotein processing have been studied in detail. However, the mechanism of cell to cell movement and the role of the putative movement protein in the process have not been investigated in any Sobemovirus thus far.

8. The objectives of the present study are as follows;
   - To over express the SeMV MP in *E.coli,* purify and determine its biophysical properties.
   - To establish the interaction MP with CP *in vitro* and delineate the domain of MP that is involved in this interaction.

- To confirm the MP-CP interaction and identify the interacting domain of MP by using Yeast two hybrid system.
- To identify interacting partners of SeMV MP among other viral encoded proteins/domains.
- To elucidate the mechanism of Cell to Cell movement in SeMV.

9. Sequence analysis showed that within Sobemovirus group the sequence identity is limited among the MPs. SeMV MP is closest to SBMV MP with 50% sequence similarity.

10. SeMV MP was cloned in plasmid pRSET C and expressed as an N terminal His-tag fusion protein in *E.coli* BL21. The expressed protein went into inclusion bodies. However, the protein could be purified under denaturation condition using 8 M urea or 6 M Gn-HCl. Since, protein obtained from 6 M Gn-HCl denaturation was more stable and soluble compared to that obtained from 8 M urea, it was used for further characterization.

11. As predicted from bioinformatics analysis, the MP was predominately α- helical as observed from CD analysis and was thermally stable. However, the protein eluted in the void volume in gel filtration chromatography suggesting that the refolded MP formed large soluble aggregates in the buffer condition used. It is well known that most of the MPs form soluble aggregates probably because of their inherent property to form M-complex or tubules for transport across plasmodesmata.

12. His-MP bound to cognate RNA in a concentration dependent manner. A study of MPs from different genera shows that, they can bind to different types of nucleic acid such as RNA or DNA. Further, most of the MPs transport nucleic acid in a sequence non-specific manner.

13. MPs also require the presence of CP for the transport of viral RNA from cell to cell in most of the cases. His-MP interacted with NV specifically in a concentration dependent manner at physiological pH. The nature of the

interaction is probably hydrophobic as NaCl upto a concentration of 1 M was unable to disrupt the interaction between the proteins.

14. Many of the MPs interact with the cytoskeleton elements for transporting their cargo across the plasmodesmata, which is an extension of the endoplasmic reticulum of the cells. Since, in most cases actin has been found to be present in side the plasmodesmata, globular actin was checked for its interaction with His-MP. However, no interaction was detected between actin and His-MP either by pull down assay or by ELISA.

15. The MP gene was also cloned into pGEX 4T1 at the *EcoR1* site and the over expressed GST-MP fusion protein was soluble, stable and could be purified in good yield.

16. EMSA experiments with GST-MP, showed that unlike His-MP, the RNP complex of GST-MP was aggregated and therefore did not enter the gel most of the times, however it also formed soluble RNP complexes. Other types of nucleic acids were used in EMSA with GST-MP. However, SeMV GST-MP was unique in being very specific towards binding its own genomic RNA and it was unable to bind to any other non-specific single stranded RNA or DNA or double stranded DNA.

17. GST-MP was also able to interact with NV confirming the results obtained with the refolded His-MP.

18. To determine the domains, which may be involved in the interaction with CP, systematic deletions were made from both N and C terminus of MP. All the GST- MP deletion mutants were soluble and could be purified by procedures similar to that used for GST-MP.

19. With respect to the interaction with NV it was observed that, whereas, C terminal deletions had no effect on the interaction, N terminal deletions affected the MP to NV interaction significantly. By the deletion of the consecutive

helices from the N terminus of the protein the NV to MP interaction was drastically decreased and was nearly abolished when 49 residues were deleted. These observations suggested that the N terminal region of the MP has the NV interacting domain.

20. Some of the MPs exhibit ATPase activity since movement phenomenon is an energy dependent process. However, SeMV MP was devoid of ATPase activity, suggesting the involvement of host factors in aiding the MP for translocating the viral genome from cell to cell.

21. Yeast 2 Hybrid study was employed to determine the interacting partners of MP with the hope that, if a protein has a known function, new proteins that bind to it bring additional components into play, ultimately contributing to the understanding of the process under study.

22. The SeMV MP and its mutant genes with a N terminal DNA binding domain were cloned in pGBK T7 vector. CP gene with a N terminal activator domain was cloned in pGAD T7 vector. The pGBKT7 and pGADT7 recombinant clones were transformed in pairs into AH109 strain to determine whether MP or the mutants and CP interact in Y2H system. This is for the first time Y2H study was undertaken for *Sobemoviruses* to determine the role of CP in cell to cell movement. This is also the first report where Y2H was employed to map interacting proteins in any *Sobemovirus*.

23. It was observed that both the proteins interacted in Y2H system, but the interaction was not as strong as observed between p53 and T antigen. All the MP mutants had still lower level of interaction with CP. This could have important significance in plant virus life cycle, since theoretically MP to CP interaction needs to be transient, as once MP transfers CP to the next cell along with the cognate nucleic acid for spread of viral infection it needs to let go of it's interacting partner CP and genome, otherwise future steps in the life cycle can not take place.

24. The deletion of amino acids from the N-terminus drastically reduced the interaction between MP and CP. 16 amino acid deletion (First helix) had no effect on MP –CP interaction. Similarly C terminal 3, 19 and 38 amino acid deletions (Figure 4.7) had no effect. These results confirmed the observations made ELISA with *E.coli* expressed proteins. But, deletion of 35 amino acids from the N terminus (first 2 helices) reduced the interaction by almost 40% and deletion of 49 amino acids (first 3 helices) by almost 50%, suggesting that the interacting domain is situated between the residues 20 to 49. This is the first report on such a study in *Sobemoviruses* with SeMV MP demonstrating that the N-terminal domain is involved in the interaction with CP.

25. Ancillary proteins are extremely important for the proper functioning of plant virus movement proteins. Previous reports with other viruses have identified many ancillary proteins which aid the MPs in their movement phenomenon. Some of these proteins are of viral origin and others are encoded by the host.

26. With a view to identify of the ancillary proteins, all the proteins expressed by SeMV genome were screened for interaction with MP in Y2H system. Interestingly among the seven proteins that SeMV encodes, MP interacts with two of the proteins, namely P10 and VPg to the highest stringency and the interaction was stronger when compared to MP-CP interaction.

27. N terminal deletions in MP drastically reduced the MP to P10 interaction with no α or β Galactosidase activity seen in the highest stringency condition, These results suggest that the MP interacts with P10 via the N terminal domain. Recently, P10 was shown to possess NTPase activity (Nair and Savithri, 2010 b). Since movement across the plasmodesmata is an active process, probably interaction of MP with P10 might result in the translocation of the M complex across plasmodesmata the energy for which might come from hydrolysis of ATP by P10.

28. MP to VPg interaction was also studied in detail. High stringency interaction was observed between MP and VPg. Interestingly, this interaction is not only

dependent on N terminal domain but also on the C terminal 19 amino acids. Further, C terminal 38 amino acid deletion from MP showed interaction between MP and VPg. It should be noted that VPg is a natively unfolded protein and the structure of MP is not known. With out further investigation it is difficult to understand why the MP fails to interact with the VPg when 19 amino acids are removed from the C terminus and starts to interact once again when additional 19 amino acids (CΔ38) are removed.

29. It may be noted that the N- terminal domain of SeMV MP interacts with all the three partners namely CP, P10 and VPg. It would be interesting to investigate if these interactions are mutually exclusive by yeast three hybrid assays.

30. The interaction of MP with VPg suggests that VPg might be the recognition factor that actually helps the MP to target its own genomic RNA for transportation across the plasmodesmata since VPg is covalently linked to the 5' end of genomic RNA.

31. To test the hypothesis that MP recognizes its own genomic RNA through VPg, the binding ability of MP to CP RNA transcript not containing covalently linked VPg and Pronase treated SeMV RNA (genomic RNA devoid of VPg) was examined. In both cases MP was unable to bind to genomic RNA.

32. This is the first time an MP has been shown to specifically interact with its genomic RNA via covalently linked VPg at the 5' end of the RNA. This would help eliminate non specific interactions with other host RNAs which do not have VPg at the 5' end. These results also corroborates with EMSA results where mutants (MP NΔ49 and MP C Δ19) which did not interact with VPg in Y2H also failed to recognize cognate genome except for MP CΔ38, which showed interaction with VPg but was unable to interact with RNA .

33. To see the localization of SeMV MP with in yeast cell, MP gene was cloned as a C terminal CFP fusion protein in pYES 2 vector and transformed in BY4743 – Ura strain. For comparison CPMV MP CFP was used. It was interesting to

observe that SeMV MP and CPMV MP formed cytosolic punctuate structures within the cell.

34. Based on the results presented in the thesis, a model for SeMV movement from cell to cell was proposed where first, the positive sense RNA genome of SeMV is translated directly in the cytosol. Once all the non structural proteins are translated with in the host cell the viral genome is replicated. MP seeks out progeny genomic RNA from the cellular milieu via the VPg at its 5' end. It also binds to P10 and CP via the N terminal domain to form the M complex that makes its way to the plasmodesmata with the energy provided by hydrolysis of ATP by P10. With the help of unknown host factors it may negotiate the plasmodesmata and move to the next cell. However, the model needs to be confirmed through *in planta* experiments using infectious SeMV cDNA clone and its mutants.